

## **Specification**

**Novel polypeptides, DNAs encoding the  
polypeptides, and Utility of the polypeptides**

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### **Technical Field**

The invention is related to novel polypeptides produced by a certain human stromal cell line and DNAs encoding the said polypeptides.

10 More particularly, the invention is related to novel polypeptides named to OAF065 $\alpha$  and OAF065 $\beta$  (called them OAF065s hereafter), a process for the preparation them, DNAs encoding the said polypeptides, a vector containing the polypeptide, a host cell transformed by the vector, antibody of the said polypeptide, a pharmaceutical composition containing the polypeptide or antibody.

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### **Technical Background**

It is known that bone marrow stromal cells form bone marrow micro environment of immunologic, hematopoietic system etc, and they produce and secret essential factors to induce of proliferation and differentiation of stem cells, e.g. IL-7, SCF, IL-11, M-CSF, G-CSF, GM-CSF, IL-6, TGF- $\beta$ , LIF etc. It is also made clear that a certain bone marrow stromal cells are related to bone metabolism (Kenneth Dorshkind Annu. Rev. Immunol. 8, 111-137. 1990). However, roles of stromal cell are not reconstituted completely from only isolated factors yet. It may suggest that existence of any factors

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which are not isolated yet.

#### Disclosure of the invention

The present inventors have directed their attention to this  
5 point and energetic research has been carried out in order to find  
novel factors (polypeptides) especially secretory and membrane  
protein which are generated by a certain stromal cells.

Until now, when a man skilled in the art intends to obtain  
a particular polypeptide or a DNA encoding it, he generally utilizes  
10 methods by confirming an intended biological activity in a tissue  
or in a cell medium, isolating and purifying the polypeptide and  
then cloning a gene or methods by "expression-cloning" with the  
guidance of the biological activity.

However, physiologically active polypeptides in living body  
15 have often many kinds of activities. Therefore, it is increasing  
that after a gene is cloned, the gene is found to be identical to  
that encoding a polypeptide already known. Generally bone marrow  
stromal cell generates only a very slight amount of a factor and  
it makes difficult to isolate and to purify the factor and to confirm  
20 its biological activity.

Recent rapid developments in techniques for constructing  
cDNAs and sequencing techniques have made it possible to quickly  
sequence a large amount of cDNAs. By utilizing these techniques,  
a process, which comprises constructing cDNAs at random,  
25 identifying the nucleotide sequences thereof, expressing novel

polypeptides encoded by them, is now in progress. Although this process is advantageous in that a gene can be cloned and information regarding its nucleotide sequence can be obtained without any biochemical or genetic analysis, the target gene can be discovered  
5 thereby only accidentally in many cases.

The present inventors have studied cloning method of genes coding proliferation and/or differentiation factors functioning in hematopoietic systems and immune systems. Focusing their attention on the fact that most of the secretory proteins such as  
10 proliferation and/or differentiation factors (for example various cytokines) and membrane proteins such as receptors thereof (hereafter these proteins will be referred to generally as secretory proteins and the like) have sequences called signal peptides in the N-termini, the inventors conducted extensive studies on a  
15 process for efficiently and selectively cloning a gene coding for a signal peptide. Finally, we have successfully invented a screening method for cDNAs having sequence encoding signal peptides, we called the method as signal sequence trap (SST) (See Japanese Patent Application No. 6-13951). We also developed yeast SST  
20 method on the same concept. By the method using yeast, genes including sequence encoding signal peptide can be identified more easily and effectively (See USP No. 5,536,637).

By using SST method, the present inventors achieved to find novel membrane proteins produced by bone marrow stromal cell and  
25 DNAs encoding them, and we then completed the invention.

The polypeptide OAF065s of the invention are not known one, when amino acid sequences of the polypeptide was compared by a computer to all known sequences in data base of Swiss Prot Release 33. It was found out that the polypeptides of the invention are type-I membrane protein and they have extracellular Cys rich region which commonly exists in the receptor family of Tumor necrosis factor (TNF) (See Fig. 1). So it was suggested that the polypeptides of the invention are novel membrane proteins which belong to TNF receptor family.

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#### **Brief Description of the Drawing**

Fig. 1 shows comparison of the amino acid sequence of the invention and that of TNF receptor family. hTNFR1 represents human necrosis factor receptor 1, hTNFR2 represents human necrosis factor receptor 2, hNGFR represents human nerve growth factor receptor, and hFas represents human Fas, in this figure.

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#### **Detailed Description**

##### **of the invention**

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The invention provides:

- 1) a polypeptide comprising an amino acid sequence shown in SEQ ID NO. 1 or NO. 5,
- 2) a DNA encoding the polypeptides described above (1),
- 3) a DNA comprising a nucleotide sequence shown in SEQ ID NO. 2 or NO. 6,

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4) a DNA comprising a nucleotide sequence shown in SEQ ID NO. 3 or NO. 7.

More particularly, the invention is concerned with a polypeptide comprising amino acid sequence shown in SEQ ID NO. 1 or 5 in substantially purified form, a homologue thereof, a fragment of the sequence and a homologue of the fragment. Further, the invention is concerned with DNAs encoding the above peptides. More particularly the invention is provided DNAs comprising nucleotide sequence shown in SEQ ID NO. 2, 3, 6 or 7, and DNA containing a fragment which is selectively hybridizing to the DNA comprising nucleotide sequence shown in SEQ ID NO. 2, 3, 6 or 7.

A polypeptide comprising amino acid sequence shown in SEQ ID NO. 1 or 5 in substantially purified form will generally comprise the polypeptide in a preparation in which more than 90%, e.g. 95%, 98% or 99% of the polypeptide in the preparation is that of the SEQ ID NO. 1 or 5. A homologue of polypeptide comprising amino acid sequence shown in SEQ ID NO. 1 or 5 will be generally at least 70%, preferably at least 80 or 90% and more preferably at least 95% homologous to the polypeptide comprising amino acid sequence shown in SEQ ID NO. 1 over a region of at least 20, preferably at least 30, for instance 40, 60 or 100 more contiguous amino acids. Such a polypeptide homologue will be referred to a polypeptide of the invention.

Generally, a fragment of polypeptide comprising amino acid sequence shown in SEQ ID NO. 1 or 5 or its homologues will be at

least 10, preferably at least 15, for example 20, 25, 30, 40, 50 or 60 amino acids in length, and are also referred to by the term "a polypeptide of the invention".

A DNA capable of selectively hybridizing to the DNA comprising nucleotide sequence shown in SEQ ID NO. 2, 3, 6 or 7 will be generally at least 70%, preferably at least 80 or 90% and more preferably at least 95% homologous to the DNA comprising nucleotide sequence shown in SEQ ID NO. 2, 3, 6 or 7 over a region of at least 20, preferably at least 30, for instance 40, 60 or 100 or more contiguous nucleotides. Such DNA will be referred to "a cDNA of the invention".

Fragments of the DNA comprising nucleotide sequence shown in SEQ ID NO. 2, 3, 6 or 7 will be at least 10, preferably at least 15, for example 20, 25, 30 or 40 nucleotides in length, and will be also referred to "a DNA of the invention" as used herein.

A further embodiment of the invention provides replication and expression vectors carrying DNA of the invention. The vectors may be, for example, plasmid, virus or phage vectors provided with an origin of replication, optionally a promoter for the expression of the said DNA and optionally a regulator of the promoter. The vector may contain one or more selectable marker genes, for example a ampicillin resistance gene. The vector may be used in vitro, for example of the production of RNA corresponding to the cDNA, or used to transfect or transfect a host cell.

A further embodiment of the invention provides host cells

transformed with the vectors for the replication and expression of the DNA of the invention, including the DNA SEQ ID NO. 2, 3, 6 or 7 or the open reading frame thereof. The cells will be chosen to be compatible with the vector and may for example be bacterial, yeast, insect or mammalian.

A further embodiment of the invention provides a method of producing a polypeptide which comprises culturing host cells of the invention under conditions effective to express a polypeptide of the invention. Preferably, in addition, such a method is carried out under conditions in which the polypeptide of the invention is expressed and then produced from the host cells.

DNA of the invention may also be inserted into the vectors described above in an antisense orientation in order to provide for the production of antisense RNA. Such antisense RNA may be used in a method of controlling the levels of a polypeptide of the invention in a cell.

The invention also provides monoclonal or polyclonal antibodies against a polypeptide of the invention. The invention further provides a process for the production of monoclonal or polyclonal antibodies to the polypeptides of the invention. Monoclonal antibodies may be prepared by common hybridoma technology using polypeptides of the invention or fragments thereof, as an immunogen. Polyclonal antibodies may also be prepared by common means which comprise inoculating host animals, for example a rat or a rabbit, with polypeptides of the invention and recovering

immune serum.

The invention also provides pharmaceutical compositions containing a polypeptide of the invention, or an antibody thereof, in association with a pharmaceutically acceptable diluent and/or carrier.

The polypeptide of the invention includes that which a part of their amino acid sequence is lacking (e.g., a polypeptide comprised of the only essential sequence for revealing a biological activity in an amino acid sequence shown in SEQ ID NO.1), that which a part of their amino acid sequence is replaced by other amino acids (e.g., those replaced by an amino acid having a similar property) and that which other amino acids are added or inserted into a part of their amino acid sequence, as well as those comprising the amino acid sequence shown in SEQ ID NO. 1 or 5.

As known well, there are one to six kinds of codon as that encoding one amino acid (for example, one kind of codon for Methioine (Met), and six kinds of codon for leucine (Leu) are known). Accordingly, the nucleotide sequence of DNA can be changed in order to encode the polypeptide having the same amino acid sequence.

The DNA of the invention, specified in (2) includes a group of every nucleotide sequences encoding polypeptides (1) shown in SEQ ID NO. 1 or 5. There is a probability that yield of a polypeptide is improved by changing a nucleotide sequence.

The DNA specified in (3) is the embodiment of the DNA shown in (2), and indicate the sequence of natural form.



The DNA shown in (4) indicates the sequence of the DNA specified in (3) with natural non-translational region.

cDNA carrying nucleotide sequence shown in SEQ ID NO. 3 is prepared by the following method:

5        Brief description of Yeast SST method (see USP No. 5,536,637) is as follows.

Yeast such as *Saccharomyces cerevisiae* should secrete invertase into the medium in order to take sucrose or raffinose as a source of energy or carbon (Invertase is an enzyme to cleave  
10 raffinose into sucrose and melibiose, sucrose into fructose and glucose.). It is known that many known mammalian signal sequence make yeast secrete its invertase. From these knowledge, SST method was developed as a screening method to find novel signal sequence which make it possible can to secrete yeast invertase from  
15 mammalian cDNA library. SST method uses yeast growth on raffinose medium as a marker. Non-secretory type invertase gene SUC2 (GENBANK Accession No. V 01311) lacking initiation codon ATG was inserted to yeast expression vector to prepare yeast SST vector pSUC2. In this expression vector, ADH promoter, ADH terminator  
20 (both were derived from AAH5 plasmid (Gammerer, Methods in Enzymol. 101, 192-201, 1983)), 2 $\mu$  ori (as a yeast replication origin), TRP1 (as a yeast selective marker), ColE1 ori (as a E. Coli replication origin) and ampicillin resistance gene (as a drug resistance marker) were inserted. Mammalian cDNA was inserted into the  
25 upstream of SUC2 gene to prepare yeast SST cDNA library. Yeast

lacking secretory type invertase, was transformed with this library. If inserted mammalian cDNA encodes a signal peptide, yeast could be survive in raffinose medium as a result of restoring secretion of invertase. Only to culture yeast colonies, prepare plasmids and  
5 determine the nucleotide sequence of the insert cDNAs, it is possible to identify novel signal peptide rapidly and easily.

Preparation of yeast SST cDNA library is as follows:

- (1) mRNA is isolated from the targeted cells, second-strand  
10 synthesis is performed by using random primer with certain restriction enzyme (enzyme I) recognition site,
- (2) double-strand cDNA is ligated to adapter containing certain restriction endonuclease (enzyme II) recognition site, differ from enzyme I, digested with enzyme I and fractionated in a appropriate  
15 size,
- (3) obtained cDNA fragment is inserted into yeast expression vector on the upstream region of invertase gene which signal peptide is deleted and the library was transformed.

20 Detailed description of each step is as follows:

- (1) mRNA is isolated from mammalian organs and cell lines stimulate them with appropriate stimulator if necessary) by known methods (Molecular Cloning (Sambrook, J., Fritsch, E. F. and Maniatis, T., Cold Spring Harbor Laboratory Press, 1989) or Current Protocol in  
25 Molecular Biology (F. M. Ausubel et al, John Wiley & Sons, Inc.)

if not remark especially).

HAS303 (human bone marrow stromal cell line: provide from Professor Keisuke Sotoyama, Dr. Makoto Aizawa of Tokyo Medical College, 1st medicine; see J. Cell. Physiol., 148, 245-251, 1991 and Experimental Hematol., 22, 482-487, 1994) and HUVEC (human umbilical vein cord endothelial cell: ATCC No. CRL-1730) are chosen as a tissue source. Double-strand cDNA synthesis using random primer is performed by known methods.

Any sites may be used as restriction endonuclease recognition site I which is linked to adapter and restriction endonuclease recognition site II which is used in step (2), if both sites are different each other. Preferably, EcoRI is used as enzyme I and XhoI as enzyme II.

In step (2), cDNA is created blunt-ends with T4 DNA polymerase, ligated enzyme II adapter and digested with enzyme I. Fragment cDNA is analyzed with agarose-gel electrophoresis (AGE) and is selected cDNA fraction ranging in size from 300 to 800 bp. As mentioned above, any enzyme may be used as enzyme II if it is not same the enzyme I.

In step (3), cDNA fragment obtained in step (2) is inserted into yeast expression vector on the upstream region of invertase gene which signal peptide is deleted. E. coli transformed with the expression vector. Many vectors are known as yeast expression plasmid vector. For example, YEp24 is also functioned in E. Coli.

Preferably pSUC2 as described above is used.

Many host E. Coli strains are known for transformation, preferably DH10B competent cell is used. Any known transformation method is available, preferably it is performed by electroporation method. Transformant is cultured by conventional methods to obtain  
5 cDNA library for yeast SST method.

However not every All of the clones do not contain cDNA fragment. Further all of the gene fragments do not encode unknown signal peptides. It is therefore necessary to screen a gene fragment encoding for an unknown signal peptide from the library.

10 Therefore, screening of fragments containing a sequence encoding an appropriate signal peptide is performed by transformation of the cDNA library into *Saccharomyces cerevisiae* (e.g. YTA455 strain) which lack invertase (it may be prepared by known methods.). Transformation of yeast is performed by known  
15 methods, e.g. lithium acetate method. Transformant is cultured in a selective medium, then transferred to a medium containing raffinose as a carbon source. Survival colonies are selected and then prepared plasmid. Survival colonies on a raffinose-medium indicates that some signal peptide of secretory protein was inserted  
20 to this clone.

Isolated positive clones is determined the nucleotide sequence. As to a cDNA encodes unknown protein, full-length clone may be isolated by using cDNA fragment as a probe and then determined to obtain full-length nucleotide sequence. These  
25 manipulation is performed by known methods.

Once the nucleotide sequences shown in SEQ ID NO. 2, 3, 6 or 7 are determined partially or preferably fully, it is possible to obtain DNA encode mammalian protein itself, homologue or subset. cDNA library or mRNA derived from mammals was screened by PCR with any synthesized oligonucleotide primers or by hybridization with any fragment as a probe. It is possible to obtain DNA encodes other mammalian homologue protein from other mammalian cDNA or genome library.

If a cDNA obtained above contains a nucleotide sequence of cDNA fragment obtained by SST (or consensus sequence thereof), it will be thought that the cDNA encodes signal peptide. So it is clear that the cDNA will be full-length or almost full.

(All signal sequences exist at N-termini of a protein and are encoded at 5'-temini of open reading frame of cDNA.)

The confirmation may be carried out by Northern analysis with the said cDNA as a probe. It is thought that the cDNA is almost complete length, if length of the cDNA is almost the same length of the mRNA obtained in the hybridizing band.

Once the nucleotide sequences shown in SEQ ID NOS. 2, 3, 6 or 7 are determined, DNAs of the invention are obtained by chemical synthesis, or by hybridization making use of nucleotide fragments which are chemically synthesized as a probe. Furthermore, DNAs of the invention are obtained in desired amount by transforming a vector that contains the DNA into a proper host, and culturing the transformant.

The polypeptides of the invention may be prepared by:

- (1) isolating and purifying from an organism or a cultured cell,
- (2) chemically synthesizing, or
- (3) using recombinant DNA technology,

5 preferably, by the method described in (3) in an industrial production.

Examples of expression system (host-vector system) for producing a polypeptide by using recombinant DNA technology are the expression systems of bacteria, yeast, insect cells and  
10 mammalian cells.

In the expression of the polypeptide, for example, in E. Coli, the expression vector is prepared by adding the initiation codon (ATG) to 5' end of a DNA encoding mature peptide, connecting the DNA thus obtained to the downstream of a proper promoter (e.g.,  
15 trp promoter, lac promoter,  $\lambda$  PL promoter, T7 promoter etc.), and then inserting it into a vector (e.g., pBR322, pUC18, pUC19 etc.) which functions in an E. coli strain.

Then, an E. coli strain (e.g., E. coli DH1 strain, E. coli JM109 strain, E. coli HB101 strain, etc.) which is transformed with  
20 the expression vector described above may be cultured in a appropriate medium to obtain the desired polypeptide. When a signal peptide of bacteria (e.g., signal peptide of pel B) is utilized, the desired polypeptide may be also released in periplasm. Furthermore, a fusion protein with other polypeptide may be also  
25 produced easily.

In the expression of the polypeptide, for example, in a mammalian cells, for example, the expression vector is prepared by inserting the DNA encoding nucleotide shown in SEQ ID NO. 3 or 7 into the downstream of a proper promoter (e.g., SV40 promoter, LTR promoter, metallothionein promoter etc.) in a proper vector (e.g., retrovirus vector, papilloma virus vector, vaccinia virus vector, SV40 vector, etc.). A proper mammalian cell (e.g., monkey COS-7 cell, Chinese hamster CHO cell, mouse L cell etc.) is transformed with the expression vector thus obtained, and then the transformant is cultured in a proper medium to get a desired polypeptide on the cell membrane. A vector described above can be inserted with deletion mutant DNA that encodes sequence, which is deleted transmembrane region from SEQ ID NOs. 3 or 7 and the expression vector can be transfected into an appropriate mammalian cell. The aimed soluble protein can be secreted into the culture medium. The polypeptide available by the way described above can be isolated and purified by conventional biochemical method.

#### **Industrial Applicability**

The polypeptide OAF065s of the invention show significant homology with a series of proteins which belong to TNF receptor family. Proteins, which belong to TNF receptor family, are type-1 membrane protein which have 3 to 6 repeated structure containing 6 Cys residues in the extracellular domain. It has been apparent that the proteins are related to proliferation, differentiation

cell death of various cells by the interaction with ligand thereof

(Craig A. Smith et. al., Cell, 76, 959-962, 1994) . For instance,

Neuronal growth factor (NGF) receptor / NGF are essential for

keeping several kinds of neuronal cells surviving, allowing

5 neuronal tubes to elongate and promoting to make neuronal

transmitters (Chao M.V., J. Neurobiol., 25, 1373-1385, 1994) .

Fas/FasL is essential for maintaining homeostasis in vivo, such as

destruction of cancer cells and removal of auto-reactive

lymphocytes via its apoptosis-inducing activity, and also relates

10 to CD4-positive T cell reduction in AIDS, fulminant hepatitis, graft

versus host disease (GVHD) after transplantation and the onset of

various autoimmune diseases (Nagata S. et. al., Science, 267,

1449-1456, 1995). CD40/CD40L is essential for activating B cells

(acceleration of growth and antibody production) via T/B cell

15 interaction (Banchereau J. et. al., Annu. Rev. Immunol., 12, 881-922,

1994). TNF receptor/TNF and lymphotoxin (LT) receptor/LT have

activities, such as growth, activation and differentiation

induction of various immune and hematopoietic cells, cytotoxicity

and growth inhibition of tumor cells, growth and activation of

20 various connective tissues (e.g., endothelial cells, fibroblasts,

osteoblasts, etc.) and viral growth inhibition, and are also

essential for the morphology or organ formation of lymphoid tissue

(Ware C.F. et al., Curr. Topics Microbiol. Immunol., 198, 175-218,

1995).



Since repetitive structures of Cys are present at three points in the extracellular domain of the polypeptide of the invention, it is obvious that this is a novel protein belonging to the TNF receptor family and exerts its activity via a ligand belonging to a known or unknown TNF family. In consequence, it is considered that the polypeptide of the invention and a cDNA molecule which encodes the polypeptide will show one or more of the effects or biological activities (including those which relates to the assays cited below) concerning differentiation, proliferation, growth, survival or cell death of hematopoietic, immune and nerve system cells, immune system functions, proliferation and growth of tumor, inflammations, bone metabolism, etc. The effects or biological activities described in relation to the polypeptide of the invention are provided by administration or use of the polypeptide or by administration or use of a cDNA molecule which encodes the polypeptide (e.g., vector suitable for gene therapy or cDNA introduction).

1) Cytokine activity and cell proliferation/differentiation activity

The polypeptide of the invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell

5 differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a  
10 convenient confirmation of cytokine activity. The activity of a polypeptide of the invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines.

15 2) Immune stimulating/suppressing activity

The polypeptide of the invention may also exhibit immune stimulating or immune suppressing activity. The polypeptide of the invention may be useful in the treatment of various immune deficiencies and disorders (including severe combined  
20 immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g. HIV) as well as bacterial or fungal infections, or may result from  
25 autoimmune disorders. More specifically, infectious diseases

causes by viral, bacterial, fungal or other infection may be treatable using the polypeptide of the invention, including infections by HIV, hepatitis viruses, herpes viruses, mycobacteria, leishmania, malaria and various fungal infections such as candida.

5 Of course, in this regard, a polypeptide of the invention may also be useful where a boost to the immune system generally would be indicated, i.e., in the treatment of cancer.

Such a polypeptide of the invention may also to be useful in the treatment of allergic reactions and conditions, such as  
10 asthma or other respiratory problems.

The polypeptide of the invention may also suppress chronic or acute inflammation, such as, for example, that associated with infection (such as septic shock or systemic inflammatory response syndrome (SIRS)), inflammatory bowel disease, Crohn's disease or  
15 resulting from over production of cytokines such as TNF or IL-1 (such as the effect demonstrated by IL- 11).

### 3) Hematopoiesis regulating activity

The polypeptide of the invention may be useful in regulation  
20 of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis.

The said biological activities are concerned with the  
25 following all or some example(s). e.g. in supporting the growth

and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility.

for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of

5 erythroid precursors and/or erythroid cells;

in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression;

10 in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions;

15 and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, 20 aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either in-vivo or ex-vivo (i.e. in conjunction with bone marrow transplantation) as normal cells or genetically manipulated for gene therapy.

25 The activity of the polypeptide of the invention may, among

other means. be measured by the following methods :

4) Tissue generation/regeneration activity

The polypeptide of the invention also may have utility in  
5 compositions used for bone, cartilage, tendon, Ligament and/or  
nerve tissue growth or regeneration, as well as for wound healing  
and tissue repair, and in the treatment of burns, incisions and ulcers.  
The polypeptide of the invention, which induces cartilage and/or  
bone growth in circumstances where bone is not normally formed,  
10 has application in the healing of bone fractures and cartilage  
damage or defects in humans and other animals. Such a preparation  
employing the polypeptide of the invention may have prophylactic  
use in closed as well as open fracture reduction and also in the  
improved fixation of artificial joints. De novo bone formation  
15 induced by an osteogenic agent contributes to the repair of  
congenital, trauma induced, or oncologic resection induced  
craniofacial defects, and also is useful in cosmetic plastic  
surgery.

The polypeptide of this invention may also be used in the  
20 treatment of periodontal disease, and in other tooth repair  
processes. Such agents may provide an environment to attract  
bone-forming cells, stimulate growth of bone-forming cells or  
induce differentiation of progenitors of bone-forming cells.  
The polypeptide of the invention may also be useful in the treatment  
25 of osteoporosis or osteoarthritis, such as through stimulation of

bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes.

Another category of tissue regeneration activity that may  
5 be attributable to the polypeptide of the invention is tendon/ligament formation. A polypeptide of the invention, which induces tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities  
10 and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/Ligament-like tissue inducing polypeptide may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to  
15 tendon or ligament tissue. De novo tendon/ligament-like tissue formation induced by a composition of the invention contributes to the repair of congenital, trauma induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments.  
20 The compositions of the invention may provide an environment to attract tendon- or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon Ligament cells or progenitors ex vivo for return in vivo  
25 to effect tissue repair. The compositions of the invention may also

be useful in the treatment of tendinitis, carpal tunnel syndrome and other tendon or ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

5       The polypeptide of the invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue. i.e. for the treatment of central and peripheral nervous system diseases and neuropathies. as well as mechanical and traumatic disorders, which involve degeneration, death or  
10 trauma to neural cells or nerve tissue. More specifically, the polypeptide of the invention may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease,  
15 Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions which may be treated in accordance with the invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies  
20 resulting from chemotherapy or other medical therapies may also be treatable using the polypeptide of the invention.

It is expected that the polypeptide of the invention may also exhibit activity for generation of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin,  
25 endothelium), muscle (smooth, skeletal or cardiac) and vascular

(including vascular endothelium) tissue, or for promoting the proliferation of cells comprising such tissues. Part of the desired effects may be by inhibition of fibrotic scarring to allow normal tissue to regenerate.

5           A polypeptide of the invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage.

10   5)       Activin/Inhibin activity

          The polypeptide of the invention may also exhibit activin- or inhibin-related activities. Inhibins are characterized by their ability to inhibit the release of follicle stimulating hormone (FSH), while activins and are characterized by their ability to stimulate  
15   the release of follicle stimulating hormone (FSH). Thus, a polypeptide of the invention alone or in heterodimers with a member of the inhibin  $\alpha$  family, may be useful as a contraceptive based on the ability of inhibins to decrease fertility in female mammals and decrease spermatogenesis in male mammals. Administration of  
20   sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the polypeptide of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin- $\beta$  group, may be useful as a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH  
25   release from cells of the anterior pituitary. See for example, USP



4,798,885. The polypeptide of the invention may also be useful for advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as cows, sheep and pigs.

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6) Chemotactic/chemokinetic activity

A polypeptide of the invention may have chemotactic or chemokinetic activity (e.g., act as a chemokine) for mammalian cells, including, for example, monocytes, neutrophils, T-cells, mast cells, eosinophils and/or endothelial cells. Chemotactic and chemokinetic proteins can be used to mobilized or attract a desired cell population to a desired site of action. Chemotactic or chemokinetic proteins provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. For example, attraction of lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such cell population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

7) Hemostatic and thrombolytic activity

The polypeptide of the invention may also exhibit hemostatic or thrombolytic activity. As a result, such a protein is expected to be useful in treatment of various coagulation disorders (including hereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events in treating wounds resulting from trauma, surgery or other causes. A protein of the invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of conditions resulting therefrom (such as, for example, infarction or stroke).

8) Receptor/ligand activity

The polypeptide of the invention may also demonstrate activity as receptors, receptor ligands or inhibitors or agonists of receptor/ligand interactions. Examples of such receptors and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell-cell interactions and their ligands (including cellular adhesion molecules (such as selectins, integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses). Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand

interaction. A polypeptide of the invention (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

5    9)      Other activity

          The polypeptide of the invention may also exhibit one or more of the following additional activities or effects: inhibiting the growth, infection or function of, or killing, infectious agents, including, bacteria, viruses, fungi and other parasites;

10    effecting (suppressing or enhancing) bodily characteristics, including, height, weight, hair color, eye color, skin, fat to lean ratio or other tissue pigmentation, or organ or body part size or shape (such as, for example, breast augmentation or diminution); effecting elimination of dietary fat, protein, carbohydrate;

15    effecting behavioral characteristics, including appetite, libido, stress, cognition (including cognitive disorders), depression and violent behaviors;

          providing analgesic effects or other pain reducing effects;

          promoting differentiation and growth of embryonic stem cells in

20    lineages other than hematopoietic lineages;

          in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases.

          The polypeptide with above activities, is suspected to have following functions by itself or interaction with its ligands or

25    receptors or association with other molecules. For example,

proliferation or cell death of B cells, T cells and/or mast cells  
 or class specific induction of B cells by promotion of class switch  
 of immunoglobulin genes; differentiation of B cells to  
 antibody-forming cells; proliferation, differentiation, or cell  
 5 death of precursors of granulocytes; proliferation,  
 differentiation, or cell death of precursors of monocytes-  
 macrophages;  
 proliferation, of up regulation or cell death of neutrophils,  
 monocytes-macrophages, eosinophils and/or basophils;  
 10 proliferation, or cell death of precursors of megakaryocytes;  
 proliferation, differentiation, or cell death of precursors of  
 neutrophils; proliferation, differentiation, or cell death of  
 precursors of T cells and B cells; promotion of production of  
 erythrocytes; sustainment of proliferation of erythrocytes,  
 15 neutrophils, eosinophils, basophils, monocytes-macrophages, mast  
 cells, precursors of megakaryocyte ; promotion of migration of  
 neutrophils, monocytes-macrophages, B cells and/or T cells;  
 proliferation or cell death of thymocytes; suppression of  
 differentiation of adipocytes; proliferation or cell death of  
 20 natural killer cells;  
 proliferation or cell death of hematopoietic stem cells;  
 suppression of proliferation of stem cells and each hematopoietic  
 precursor cells; promotion of differentiation from mesenchymal stem  
 cells to osteoblasts or chondrocytes, proliferation or cell death  
 25 of mesenchymal stem cells, osteoblasts or chondrocytes and

promotion of bone absorption by activation of osteoclasts and  
promotion of differentiation from monocytes to osteoclasts.

This peptide is also suspected to function to nervous system,  
5 so expected to have functions below; differentiation to kinds of  
neurotransmitter-responsive neurons, survival or cell death of  
these cells; promotion of proliferation or cell death of glial  
cells; spread of neural dendrites; survival or cell death of  
gangriocytes; proliferation, promotion of differentiation, or cell  
10 death of astrocytes; proliferation or survival of peripheral  
neurons; proliferation or cell death of Schwann cells;  
proliferation, survival or cell death of motoneurons.

Furthermore, in the process of development of early  
embryonic, this polypeptide is expected to promote or inhibit the  
15 organogenesis of epidermis, brain, backbone, and nervous system  
by induction of ectoderm, that of notochord connective tissues (bone,  
muscle, tendon), hemocytes, heart, kidney, and genital organs by  
induction of mesoderm, and that of digestive apparatus (stomach,  
intestine, liver, pancreas), respiratory apparatus (lung, trachea)  
20 by induction of endoderm. In adult, also, this polypeptide is  
thought to proliferate or inhibit the above organs.

Therefore, this polypeptide itself is expected to be used  
as an agent for the prevention or treatment of disease of progression  
or suppression of immune, nervous, or bone metabolic function,  
25 hypoplasia or overgrowth of hematopoietic cells: inflammatory

disease (rheumatism, ulcerative colitis, etc.), decrease of hematopoietic stem cells after bone marrow transplantation, decrease of leukocytes, platelets, B-cells, or T-cells after radiation exposure or chemotherapeutic dosage against cancer or leukemia, anemia, infectious disease, cancer, leukemia, AIDS, bone metabolic disease(osteoporosis etc.), various degenerative disease (Alzheimer's disease, multiple sclerosis, etc.), or nervous lesion.

In addition, since this polypeptide is thought to induce the differentiation or growth of organs derived from ectoderm, mesoderm, and endoderm, this polypeptide is expected to be an agent for tissue repair (epidermis, bone, muscle, tendon, heart, kidney, stomach, intestine, liver, pancreas, lung, and trachea, etc.).

Quantitation of the polypeptide of the invention in the body can be performed using polyclonal or monoclonal antibodies against the polypeptide of the invention. It can be used the study of relationship between this polypeptide and disease or diagnosis of disease, and so on. Polyclonal and monoclonal antibodies can be prepared using this polypeptide or its fragment as an antigen by conventional methods.

Identification, purification or molecular cloning of known or unknown proteins which bind the polypeptide of the invention (preferably polypeptide of extracellular domain) can be performed using the polypeptide of the invention by, for example, preparation of the affinity-column.

Identification of the downstream signal transmission molecules which interact with the polypeptide of the invention in cytoplasm and molecular cloning of the gene can be performed: by west-western method using the polypeptide of the invention  
5 (preferably polypeptide of transmembrane region or intracellular domain) or  
by yeast two-hybrid system using the cDNA (preferably cDNA encoding transmembrane region or cytoplasmic domain of the polypeptide).

Agonists/antagonists of this receptor polypeptide and  
10 inhibitors between receptor and signal transduction molecules can be screened using the polypeptide of the invention.

cDNAs of the invention are useful not only the important and essential template for the production of the polypeptide of the invention which is expected to be largely useful, but also be useful  
15 for diagnosis or therapy (for example, treatment of gene lacking, treatment to stop the expression of the polypeptide by antisense DNA (RNA)). Genomic DNA may be isolated with the cDNA of the invention, as a probe. As the same manner, a human gene encoding which can be highly homologous to the cDNA of the invention, that  
20 is, which encodes a polypeptide highly homologous to the polypeptide of the invention and a gene of animals excluding mouse which can be highly homologous to the cDNA of the invention, also may be isolated.

[Application to medicaments]

The polypeptide of the invention or the antibody specific for the polypeptide of the invention is administered systemically or topically and in general orally or parenterally for preventing or treating diseases related to incomplete growth or abnormal growth of hematopoietic system cells, acceleration or reduction of nerve system functions or acceleration or reduction of immune system functions, such as inflammatory diseases (e.g., rheumatoid, ulcerative colitis, etc.), cytopenia of hematopoietic stem cells after bone marrow transplantation, cytopenia of leukocytes, platelets, B cells or T cells after radiation treatment or after administration of a chemotherapeutic agent, anemia, infectious diseases, cancer, leukemia, AIDS, and various degenerative diseases (e.g., Alzheimer's disease, multiple sclerosis, etc.), or nerve damage, for preventing or treating metabolic disorder of bones (e.g., osteoporosis, etc.), or for repairing tissues. Oral administration, intravenous injection and intraventricular administration are preferred.



The doses to be administered depend upon age, body weight, symptom, desired therapeutic effect, route of administration, and duration of the treatment etc. In human adults, one dose per person is generally between 100  $\mu$ g and 100 mg, by oral administration, up to several times per day, and between 10  $\mu$ g and 100 mg, by parenteral administration up to several times per day.

As mentioned above, the doses to be used depend upon various conditions. Therefore, there are cases in which doses lower than or greater than the ranges specified above may be used.

The compounds of the invention, may be administered as solid compositions, liquid compositions or other compositions for oral administration, as injections, liniments or suppositories etc. for parenteral administration.

Solid compositions for oral administration include compressed tablets, pills, capsules, dispersible powders, granules. Capsules include soft or hard capsules.

In such compositions, one or more of the active compound(s) is or are admixed with at least one inert diluent (such as lactose, mannitol, glucose, hydroxypropyl cellulose, microcrystalline cellulose, starch, polyvinylpyrrolidone, magnesium metasilicate aluminate, etc.). The compositions may also comprise, as is normal practice, additional substances other than inert diluents: e.g. lubricating agents (such as magnesium stearate etc.),

disintegrating agents (such as cellulose calcium glycolate, etc.), stabilizing agents (such as human serum albumin, lactose etc.), and assisting agents for dissolving (such as arginine, asparaginic acid etc.).

5       The tablets or pills may, if desired, be coated with a film of gastric or enteric materials (such as sugar, gelatin, hydroxypropyl cellulose or hydroxypropylmethyl cellulose phthalate, etc.), or be coated with more than two films. And then, coating may include containment within capsules of absorbable  
10 materials such as gelatin.

Liquid compositions for oral administration include pharmaceutically-acceptable emulsions, solutions, syrups and elixirs. In such compositions, one or more of the active compound(s) is or are contained in inert diluent(s) commonly used  
15 (purified water, ethanol etc.). Besides inert diluents, such compositions may also comprise adjuvants (such as wetting agents, suspending agents, etc.), sweetening agents, flavoring agents, perfuming agents, and preserving agents.

Other compositions for oral administration include spray  
20 compositions which may be prepared by known methods and which comprise one or more of the active compound(s). Spray compositions may comprise additional substances other than inert diluents: e.g. stabilizing agents (sodium sulfite etc.), isotonic buffer (sodium chloride, sodium citrate, citric acid, etc.). For preparation of  
25 such spray compositions, for example, the method described in the

United States Patent No. 2,868,691 or 3,095,355 (herein incorporated in their entireties by reference) may be used.

5       Injections for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions and emulsions. In such compositions, one or more active compound(s) is or are admixed with at least one inert aqueous diluent(s) (distilled water for injection, physiological salt solution, etc.) or inert non-aqueous diluents(s) (propylene glycol, polyethylene glycol, olive oil, ethanol, POLYSOLBATE 80 TM, etc.).

10       Injections may comprise additional compound other than inert diluents: e.g. preserving agents, wetting agents, emulsifying agents, dispersing agents, stabilizing agent (such as human serum albumin, lactose, etc.), and assisting agents such as assisting agents for dissolving (arginine, asparaginic acid, etc.).

#### Best Mode carrying out the Invention

The invention are illustrated by the following examples, but not limit the invention.

#### Example

20       Total RNA was prepared from human bone marrow stromal cell line HAS303 (provided from Professor Keisuke Sotoyama, Dr. Makoto Aizawa, first medicine, Tokyo Medical College; See J. Cell. Physiol., 148 : 245-251 (1991) and Experimental Hematol., 22 : 482-487(1994)) by TRIzol reagent (Trade Mark, GIBCOBRL). Poly(A)RNA was purified  
25       from the total RNA by mRNA purification kit (commercial name,

Pharmacia).

Double strand cDNA was synthesized by SuperScript Plasmid System for cDNA Synthesis and Plasmid Cloning (brand name, GIBCOBRL) with above poly(A)RNA as template and random 9mer as primer which  
5 was containing XhoI site:

SEQ ID NO. 9

5'-CGA TTG AAT TCT AGA CCT GCC TCG AGN NNN NNN NN-3'

cDNA was ligated EcoRI adapter by DNA ligation kit ver.2 (trade  
10 name, Takara Shuzo; this kit was used in all ligating steps hereafter.) and digested by XhoI. cDNAs were separated by agarose-gel electrophoresis. 300 - 800 bp cDNAs were isolated and were ligated to EcoRI/NotI site of pSUC2 (see US 5,536,637). E. Coli DH10B strain were transformed by pSUC2 with electroporation  
15 to obtain yeast SST cDNA library.

Plasmids of the cDNA library were prepared. Yeast YTK12 strain were transformed by the plasmids with lithium acetate method (Current Protocols In Molecular Biology 13.7.1). The transformed yeast were plated on triptphan-free medium (CMD-Try medium) for  
20 selection. The plate was incubated for 48 hour at 30 °C. Replica of the colony which is obtained by Accutran Replica Plater (trade name, Schleicher & Schuell) were place YPR plate containing raffinose for carbon source, and the plate was incubated for 14 days at 30 °C. After 3 days, each colony appeared was streaked on  
25 YPR plate again. The plates were incubated for 48 hours at 30 °C.

Single colony was inoculated to YPR medium and was incubated for 48 hours at 30 °C. Then plasmids were prepared. Insert cDNA was amplified by PCR with two kind primers which exist end side of cloning site on pSUC2 (sense strand primers were biotinylated).

5 Biotinylated single strand of cDNAs were purified with Dynabeads (trade name, DYNAL) and determined the nucleotide sequences. Sequencing was performed by Dye Terminator Cycle Sequencing Ready Reaction with DNA Sequencing kit (trade name, Applied Biosystems Inc.) and sequence was determined by DNA sequencer 373 (Applied  
10 Biosystems Inc.). All sequencing hereafter was carried with this method.

The clone named OAF065 is not registered on databases by homology search of nucleotide sequence and deduced amino acid sequence and so it is cleared that the sequence is novel one. We  
15 confirmed that OAF065 contains signal peptide in view of function and structure, by comparison with known peptide which has signal peptide and deduced amino acid sequence. Full length cDNA of OAF065 was isolated by 3'-RACE(Rapid Amplification of cDNA End). Marathon cDNA Amplification Kit(trade name, Clontech) was used in 3'-RACE.  
20 Adaptor-ligated double stranded cDNA was prepared from poly(A)RNA of HAS303 in line with the method of the kit. OAF065 specific primer F3 (28mer):

SEQ ID NO. 10

5'-AGA AAG ATG GCT TTA AAA GTG CTA CTA G-3'

which included a deduced initiation ATG codon region based on the information of nucleotide sequence by SST was prepared. PCR was performed with the said primer and adapter primer attached in the kit. Two kinds of cDNAs (4.0 kb and 1.5 kb) were amplified and 4.0  
5 kb-cDNA was named OAF065 $\alpha$  and 1.5 kb-cDNA was named OAF065 $\beta$ .

Two kinds cDNAs were separated with agarose-gel electrophoresis, and to pT7 Blue-2 T-Vector (trade name, Novagen), ligated in and transformed to E. Coli DH5 $\alpha$  and then plasmid was prepared. Nucleotide sequences of 5'-end were determined, and the  
10 existence of nucleotide sequence OAF065 specific primer F3 were confirmed in both nucleotide sequences. 5'-End nucleotide sequence (ca 1.7 kb) of OAF065 $\alpha$  and full length nucleotide sequence of OAF065 $\beta$  were determined and then obtained sequences shown in SEQ ID NOs 3 and 7. Open reading frame was searched and deduced  
15 amino acid sequences shown in SEQ ID NO. 1 and 5 were obtained.

Compared with the nucleotide sequences of OAF065 $\alpha$  and OAF065 $\beta$ , nucleotide sequences from 1 to 1290 base were completely same, but sequences downstream from 1291 base had no homology each other. Compared with amino acid sequences of OAF065 $\alpha$  and OAF065 $\beta$ ,  
20 amino acids from 1 to 415 in N-termini were completely same, only two amino acids in C-termini of OAF065 $\alpha$  were replaced to 8 amino acids (Val Arg Gln Arg Leu Gly Ser Leu) in the sequence of OAF065 $\beta$ . It was revealed that OAF065 $\alpha$  and OAF065 $\beta$  were novel type-I membrane proteins by hydrophobicity analysis and that the extracellular  
25 region and the transmembrane region of both sequences were

consistant.

The polypeptide OAF065 $\alpha$  and OAF065 $\beta$  of the invention are not known one, when amino acid sequences of the polypeptide was compared by a computer to all known sequences in data base of Swiss Prot  
5 Release 33. Extracellular Cys rich region which commonly exists in the TNF receptor family was identified in the polypeptide of the invention.

That is, compared with amino acid sequences of the polypeptide of the invention (OAF065s) and other members of TNF  
10 receptor family i.e. human necrosis factor receptor 1 (hTNFR1), human necrosis factor receptor 2 (hTNFR2), human nerve growth factor receptor (hNGFR), and human Fas (hFas), it was revealed that the polypeptides (OAF065s) of the invention are type-I membrane protein and they have extracellular Cys rich region which commonly exists  
15 in the TNF (Tumor necrosis factor) receptor family in Fig. 1.

Therefore, it was confirmed that the polypeptides OAF065 $\alpha$  and OAF065 $\beta$  of the invention are novel membrane proteins which belong to the TNF receptor family.

# SEQUENCE LIST

SEQ ID NO.: 1

Length: 417 amino acid

Type: amino acid

5 Topology: linear

Molecule type: protein

Sequence

Met Ala Leu Lys Val Leu Leu Glu Gln Glu Lys Thr Phe Phe Thr Leu

1 5 10 15

10 Leu Val Leu Leu Gly Tyr Leu Ser Cys Lys Val Thr Cys Glu Thr Gly

20 25 30

Asp Cys Arg Gln Gln Glu Phe Arg Asp Arg Ser Gly Asn Cys Val Pro

35 40 45

Cys Asn Gln Cys Gly Pro Gly Met Glu Leu Ser Lys Glu Cys Gly Phe

15 50 55 60

Gly Tyr Gly Glu Asp Ala Gln Cys Val Thr Cys Arg Leu His Arg Phe

65 70 75 80

Lys Glu Asp Trp Gly Phe Gln Lys Cys Lys Pro Cys Leu Asp Cys Ala

85 90 95

20 Val Val Asn Arg Phe Gln Lys Ala Asn Cys Ser Ala Thr Ser Asp Ala

100 105 110

Ile Cys Gly Asp Cys Leu Pro Gly Phe Tyr Arg Lys Thr Lys Leu Val

115 120 125

Gly Phe Gln Asp Met Glu Cys Val Pro Cys Gly Asp Pro Pro Pro Pro

25 130 135 140



	Tyr	Glu	Pro	His	Cys	Ala	Ser	Lys	Val	Asn	Leu	Val	Lys	Ile	Ala	Ser
	145					150					155					160
	Thr	Ala	Ser	Ser	Pro	Arg	Asp	Thr	Ala	Leu	Ala	Ala	Val	Ile	Cys	Ser
					165					170					175	
5	Ala	Leu	Ala	Thr	Val	Leu	Leu	Ala	Leu	Leu	Ile	Leu	Cys	Val	Ile	Tyr
					180					185					190	
	Cys	Lys	Arg	Gln	Phe	Met	Glu	Lys	Lys	Pro	Ser	Trp	Ser	Leu	Arg	Ser
					195					200					205	
	Gln	Asp	Ile	Gln	Tyr	Asn	Gly	Ser	Glu	Leu	Ser	Cys	Leu	Asp	Pro	Arg
10		210				215						220				
	Gln	Leu	His	Glu	Tyr	Ala	His	Arg	Ala	Cys	Cys	Gln	Cys	Arg	Arg	Asp
	225					230						235				240
	Ser	Val	Gln	Thr	Cys	Gly	Pro	Val	Arg	Leu	Leu	Pro	Ser	Met	Cys	Cys
					245					250					255	
15	Glu	Glu	Ala	Cys	Ser	Pro	Asn	Pro	Ala	Thr	Leu	Gly	Cys	Gly	Val	His
					260					265					270	
	Ser	Ala	Ala	Ser	Leu	Gln	Ala	Arg	Asn	Ala	Gly	Pro	Ala	Gly	Glu	Met
					275					280					285	
	Val	Pro	Thr	Phe	Phe	Gly	Ser	Leu	Thr	Gln	Ser	Ile	Cys	Gly	Glu	Phe
20		290				295						300				
	Ser	Asp	Ala	Trp	Pro	Leu	Met	Gln	Asn	Pro	Met	Gly	Gly	Asp	Asn	Ile
	305					310						315				320
	Ser	Phe	Cys	Asp	Ser	Tyr	Pro	Glu	Leu	Thr	Gly	Glu	Asp	Ile	His	Ser
					325					330					335	
25	Leu	Asn	Pro	Glu	Leu	Glu	Ser	Ser	Thr	Ser	Leu	Asp	Ser	Asn	Ser	Ser

	340	345	350
	Gln Asp Leu Val Gly Gly Ala Val Pro Val Gln Ser His Ser Glu Asn		
	355	360	365
	Phe Thr Ala Ala Thr Asp Leu Ser Arg Tyr Asn Asn Thr Leu Val Glu		
5	370	375	380
	Ser Ala Ser Thr Gln Asp Ala Leu Thr Met Arg Ser Gln Leu Asp Gln		
	385	390	395
	Glu Ser Gly Ala Ile Ile His Pro Ala Thr Gln Thr Ser Leu Gln Glu		
	405	410	415
10	Ala		

SEQ ID NO.: 2

Length: 1269 base pairs

Type: nucleic acid

15 Strandness: single

Topology: linear

Molecule type: cDNA to mRNA

Sequence

20	ATGGCTTTAA AAGTGCTACT AGAACAAGAG AAAACGTTTT TCACTCTTTT AGTATTACTA	60
	GGCTATTTGT CATGTAAAGT GACTTGTGAA ACAGGAGACT GTAGACAGCA AGAATTCAGG	120
	GATCGGTCTG GAAACTGTGT TCCCTGCAAC CAGTGTGGGC CAGGCATGGA GTTGTCTAAG	180
	GAATGTGGCT TCGGCTATGG GGAGGATGCA CAGTGTGTGA CGTGCCGGCT GCACAGGTTC	240
	AAGGAGGACT GGGGCTTCCA GAAATGCAAG CCCTGTCTGG ACTGCGCAGT GGTGAACCGC	300
25	TTTCAGAAGG CAAATTGTTC AGCCACCAGT GATGCCATCT GCGGGGACTG CTTGCCAGGA	360

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TTTTATAGGA AGACGAAACT TGTCGGCTTT CAAGACATGG AGTGTGTGCC TTGTGGAGAC 420
CCTCCTCCTC CTTACGAACC GCACTGTGCC AGCAAGGTCA ACCTCGTGAA GATCGCGTCC 480
ACGGCCTCCA GCCCACGGGA CACGGCGCTG GCTGCCGTTA TCTGCAGCGC TCTGGCCACC 540
GTCCTGCTGG CCCTGCTCAT CCTCTGTGTC ATCTATTGTA AGAGACAGTT TATGGAGAAG 600
5 AAACCCAGCT GGTCTCTGCG GTCACAGGAC ATTCAGTACA ACGGCTCTGA GCTGTCGTGT 660
CTTGACAGAC CTCAGCTCCA CGAATATGCC CACAGAGCCT GCTGCCAGTG CCGCCGTGAC 720
TCAGTGCAGA CCTGCGGGCC GGTGCGCTTG CTCCCATCCA TGTGCTGTGA GGAGGCCTGC 780
AGCCCCAACC CGGCGACTCT TGGTTGTGGG GTGCATTCTG CAGCCAGTCT TCAGGCAAGA 840
AACGCAGGCC CAGCCGGGGA GATGGTGCCG ACTTTCTTCG GATCCCTCAC GCAGTCCATC 900
10 TGTGGCGAGT TTTCAGATGC CTGGCCTCTG ATGCAGAATC CCATGGGTGG TGACAACATC 960
TCTTTTGTG ACTCTTATCC TGAATCACT GGAGAAGACA TTCATTCTCT CAATCCAGAA 1020
CTTGAAAGCT CAACGTCTTT GGATTCAAAT AGCAGTCAAG ATTTGGTTGG TGGGGCTGTT 1080
CCAGTCCAGT CTCATTCTGA AAACCTTACA GCAGCTACTG ATTTATCTAG ATATAACAAC 1140
ACACTGGTAG AATCAGCATC AACTCAGGAT GCACTAACTA TGAGAAGCCA GCTAGATCAG 1200
15 GAGAGTGGCG CTATCATCCA CCCAGCCACT CAGACGTCCC TCCAGGTAAG GCAGCGACTG 1260
GGTTCCTG 1269

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SEQ ID NO.: 3

length: 1704 base pairs

20 Type: nucleic acid

Strandness: single

Topology: linear

Molecule type: cDNA to mRNA

Sequence

GGGAACGTAG AACTCTCCAA CAATAAATAC ATTTGATAAG AAAGATGGCT TTAAAAGTGC 60  
TACTAGAACA AGAGAAAACG TTTTCTACTC TTTTAGTATT ACTAGGCTAT TTGTCATGTA 120  
AAGTGACTTG TGAAACAGGA GACTGTAGAC AGCAAGAATT CAGGGATCGG TCTGGAAACT 180  
GTGTTCCCTG CAACCAGTGT GGGCCAGGCA TGGAGTTGTC TAAGGAATGT GGCTTCGGCT 240  
5 ATGGGGAGGA TGCACAGTGT GTGACGTGCC GGCTGCACAG GTTCAAGGAG GACTGGGGCT 300  
TCCAGAAATG CAAGCCCTGT CTGGACTGCG CAGTGGTGAA CCGCTTTCAG AAGGCAAATT 360  
GTTCAGCCAC CAGTGATGCC ATCTGCGGGG ACTGCTTGCC AGGATTTTAT AGGAAGACGA 420  
AACTTGTCGG CTTTCAAGAC ATGGAGTGTG TGCCTTGTGG AGACCCTCCT CCTCCTTACG 480  
AACCGCACTG TGCCAGCAAG GTCAACCTCG TGAAGATCGC GTCCACGGCC TCCAGCCCAC 540  
10 GGGACACGGC GCTGGCTGCC GTTATCTGCA GCGCTCTGGC CACCGTCCTG CTGGCCCTGC 600  
TCATCCTCTG TGTCATCTAT TGTAAGAGAC AGTTTATGGA GAAGAAACCC AGCTGGTCTC 660  
TGCGGTCACA GGACATTAG TACAACGGCT CTGAGCTGTC GTGTCTTGAC AGACCTCAGC 720  
TCCACGAATA TGCCACAGA GCCTGCTGCC AGTGCCGCCG TGAATCAGTG CAGACCTGCG 780  
GGCCGGTGCG CTTGCTCCA TCCATGTGCT GTGAGGAGGC CTGCAGCCCC AACC CGGCGA 840  
15 CTCTTGGTTG TGGGGTGCAT TCTGCAGCCA GTCTTCAGGC AAGAAACGCA GGCCAGCCG 900  
GGGAGATGGT GCCGACTTTC TTCGGATCCC TCACGCAGTC CATCTGTGGC GAGTTTTTCTCAG 960  
ATGCCTGGCC TCTGATGCAG AATCCCATGG GTGGTGACAA CATCTCTTTT TGTGACTCTT 1020  
ATCCTGAACT CACTGGAGAA GACATTCATT CTCTCAATCC AGAACTTGAA AGCTCAACGT 1080  
CTTTGGATTG AAATAGCAGT CAAGATTTGG TTGGTGGGGC TGTTCCAGTC CAGTCTCATT 1140  
20 CTGAAAACCTT TACAGCAGCT ACTGATTTAT CTAGATATAA CAACACACTG GTAGAATCAG 1200  
CATCAACTCA GGATGACTA ACTATGAGAA GCCAGCTAGA TCAGGAGAGT GGCGCTATCA 1260  
TCCACCCAGC CACTCAGACG TCCCTCCAGG AAGCTTAAAG AACCTGCTTC TTTCTGCAGT 1320  
AGAAGCGTGT GCTGGAACCC AAAGAGTACT CCTTTGTTAG GCTTATGGAC TGAGCAGTCT 1380  
GGACCTTGCA TGGCTTCTGG GGCAAAAATA AATCTGAACC AAAGTACGG CATTTGAAGC 1440  
25 CTTTCAGCCA GTTGCTTCTG AGCCAGACCA GCTGTAAGCT GAAACCTCAA TGAATAACAA 1500

GAAAAGACTC CAGGCCGACT CATGATACTC TGCATCTTTC CTACATGAGA AGCTTCTCTG 1560  
 CCACAAAAGT GACTTCAAAG ACGGATGGGT TGAGCTGGCA GCCTATGAGA TTGTGGACAT 1620  
 ATAACAAGAA ACAGAAATGC CCTCATGCTT ATTTTCATGG TGATTGTGGT TTTACAAGAC 1680  
 TGAAGACCCA GAGTATACTT TTTC 1704

5

SEQ ID NO.: 4

Length: 1704 base pairs

Type: nucleic acid

Strandness: single

10 Topology: linear

Molecule type: cDNA to mRNA

Original source:

Organism: Homo Sapiens

Cell line: HAS303

15 Feature

Name/Key: CDS

Location: 45..1295

Identification method: P

Name/Key: sig peptide

20 Location: 45..119

Identification method: S

Name/Key: mat peptide

Location: 120..1295

Identification method: S

25 Sequecne

GGGAACGTAG AACTCTCCAA CAATAAATAC ATTTGATAAG AAAG ATG GCT TTA AAA 56

Met Ala Leu Lys

-25

5 GTG CTA CTA GAA CAA GAG AAA ACG TTT TTC ACT CTT TTA GTA TTA CTA 104

Val Leu Leu Glu Gln Glu Lys Thr Phe Phe Thr Leu Leu Val Leu Leu

-20

-15

-10

GGC TAT TTG TCA TGT AAA GTG ACT TGT GAA ACA GGA GAC TGT AGA CAG 152

Gly Tyr Leu Ser Cys Lys Val Thr Cys Glu Thr Gly Asp Cys Arg Gln

10 -5

1

5

10

CAA GAA TTC AGG GAT CGG TCT GGA AAC TGT GTT CCC TGC AAC CAG TGT 200

Gln Glu Phe Arg Asp Arg Ser Gly Asn Cys Val Pro Cys Asn Gln Cys

15

20

25

GGG CCA GGC ATG GAG TTG TCT AAG GAA TGT GGC TTC GGC TAT GGG GAG 248

15 Gly Pro Gly Met Glu Leu Ser Lys Glu Cys Gly Phe Gly Tyr Gly Glu

30

35

40

GAT GCA CAG TGT GTG ACG TGC CGG CTG CAC AGG TTC AAG GAG GAC TGG 296

Asp Ala Gln Cys Val Thr Cys Arg Leu His Arg Phe Lys Glu Asp Trp

45

50

55

20 GGC TTC CAG AAA TGC AAG CCC TGT CTG GAC TGC GCA GTG GTG AAC CGC 344

Gly Phe Gln Lys Cys Lys Pro Cys Leu Asp Cys Ala Val Val Asn Arg

60

65

70

75

TTT CAG AAG GCA AAT TGT TCA GCC ACC AGT GAT GCC ATC TGC GGG GAC 392

Phe Gln Lys Ala Asn Cys Ser Ala Thr Ser Asp Ala Ile Cys Gly Asp

25

80

85

90

	TGC TTG CCA GGA TTT TAT AGG AAG ACG AAA CTT GTC GGC TTT CAA GAC	440
	Cys Leu Pro Gly Phe Tyr Arg Lys Thr Lys Leu Val Gly Phe Gln Asp	
	95 100 105	
	ATG GAG TGT GTG CCT TGT GGA GAC CCT CCT CCT CCT TAC GAA CCG CAC	488
5	Met Glu Cys Val Pro Cys Gly Asp Pro Pro Pro Pro Tyr Glu Pro His	
	110 115 120	
	TGT GCC AGC AAG GTC AAC CTC GTG AAG ATC GCG TCC ACG GCC TCC AGC	536
	Cys Ala Ser Lys Val Asn Leu Val Lys Ile Ala Ser Thr Ala Ser Ser	
	125 130 135	
10	CCA CGG GAC ACG GCG CTG GCT GCC GTT ATC TGC AGC GCT CTG GCC ACC	584
	Pro Arg Asp Thr Ala Leu Ala Ala Val Ile Cys Ser Ala Leu Ala Thr	
	140 145 150 155	
	GTC CTG CTG GCC CTG CTC ATC CTC TGT GTC ATC TAT TGT AAG AGA CAG	632
	Val Leu Leu Ala Leu Leu Ile Leu Cys Val Ile Tyr Cys Lys Arg Gln	
15	160 165 170	
	TTT ATG GAG AAG AAA CCC AGC TGG TCT CTG CGG TCA CAG GAC ATT CAG	680
	Phe Met Glu Lys Lys Pro Ser Trp Ser Leu Arg Ser Gln Asp Ile Gln	
	175 180 185	
	TAC AAC GGC TCT GAG CTG TCG TGT CTT GAC AGA CCT CAG CTC CAC GAA	728
20	Tyr Asn Gly Ser Glu Leu Ser Cys Leu Asp Rro Arg Gln Leu His Glu	
	190 195 200	
	TAT GCC CAC AGA GCC TGC TGC CAG TGC CGC CGT GAC TCA GTG CAG ACC	776
	Tyr Ala His Arg Ala Cys Cys Gln Cys Arg Arg Asp Ser Val Gln Thr	
	205 210 215	
25	TGC GGG CCG GTG CGC TTG CTC CCA TCC ATG TGC TGT GAG GAG GCC TGC	824

	Cys Gly Pro Val Arg Leu Leu Pro Ser Met Cys Cys Glu Glu Ala Cys	
	220                      225                      230                      235	
	AGC CCC AAC CCG GCG ACT CTT GGT TGT GGG GTG CAT TCT GCA GCC AGT	872
	Ser Pro Asn Pro Ala Thr Leu Gly Cys Gly Val His Ser Ala Ala Ser	
5	240                      245                      250	
	CTT CAG GCA AGA AAC GCA GGC CCA GCC GGG GAG ATG GTG CCG ACT TTC	920
	Leu Gln Ala Arg Asn Ala Gly Pro Ala Gly Glu Met Val Pro Thr Phe	
	255                      260                      265	
	TTC GGA TCC CTC ACG CAG TCC ATC TGT GGC GAG TTT TCA GAT GCC TGG	968
10	Phe Gly Ser Leu Thr Gln Ser Ile Cys Gly Glu Phe Ser Asp Ala Trp	
	270                      275                      280	
	CCT CTG ATG CAG AAT CCC ATG GGT GGT GAC AAC ATC TCT TTT TGT GAC	1016
	Pro Leu Met Gln Asn Pro Met Gly Gly Asp Asn Ile Ser Phe Cys Asp	
	285                      290                      295	
15	TCT TAT CCT GAA CTC ACT GGA GAA GAC ATT CAT TCT CTC AAT CCA GAA	1064
	Ser Tyr Pro Glu Leu Thr Gly Glu Asp Ile His Ser Leu Asn Pro Glu	
	300                      305                      310                      315	
	CTT GAA AGC TCA ACG TCT TTG GAT TCA AAT AGC AGT CAA GAT TTG GTT	1112
	Leu Glu Ser Ser Thr Ser Leu Asp Ser Asn Ser Ser Gln Asp Leu Val	
20	320                      325                      330	
	GGT GGG GCT GTT CCA GTC CAG TCT CAT TCT GAA AAC TTT ACA GCA GCT	1160
	Gly Gly Ala Val Pro Val Gln Ser His Ser Glu Asn Phe Thr Ala Ala	
	335                      340                      345	
	ACT GAT TTA TCT AGA TAT AAC AAC ACA CTG GTA GAA TCA GCA TCA ACT	1208
25	Thr Asp Leu Ser Arg Tyr Asn Asn Thr Leu Val Glu Ser Ala Ser Thr	



	350	355	360	
	CAG GAT GCA CTA ACT ATG AGA AGC CAG CTA GAT CAG GAG AGT GGC GCT	1256		
	Gln Asp Ala Leu Thr Met Arg Ser Gln Leu Asp Gln Glu Ser Gly Ala			
	365	370	375	
5	ATC ATC CAC CCA GCC ACT CAG ACG TCC CTC CAG GAA GCT TAAAGAACCT	1305		
	Ile Ile His Pro Ala Thr Gln Thr Ser Leu Gln Glu Ala			
	380	385	390	
	GCTTCTTTCT GCAGTAGAAG CGTGTGCTGG AACCCAAAGA GTACTCCTTT GTTAGGCTTA	1365		
	TGGACTGAGC AGTCTGGACC TTGCATGGCT TCTGGGGCAA AAATAAATCT GAACCAAACCT	1425		
10	GACGGCATTG GAAGCCTTTC AGCCAGTTGC TTCTGAGCCA GACCAGCTGT AAGCTGAAAC	1485		
	CTCAATGAAT AACAAGAAAA GACTCCAGGC CGACTCATGA TACTCTGCAT CTTTCCTACA	1545		
	TGAGAAGCTT CTCTGCCACA AAAGTGACTT CAAAGACGGA TGGGTTGAGC TGGCAGCCTA	1605		
	TGAGATTGTG GACATATAAC AAGAAACAGA AATGCCCTCA TGCTTATTTT CATGGTGATT	1665		
	GTGGTTTTAC AAGACTGAAG ACCCAGAGTA TACTTTTTC	1704		

15

SEQ ID NO.: 5

Length: 423 amino acids

Type: amino acid

20 Topology: linear

Molecule type: protein

Sequence

Met Ala Leu Lys Val Leu Leu Glu Gln Glu Lys Thr Phe Phe Thr Leu

25	1	5	10	15
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Leu Val Leu Leu Gly Tyr Leu Ser Cys Lys Val Thr Cys Glu Thr Gly  
                     20                    25                    30  
 Asp Cys Arg Gln Gln Glu Phe Arg Asp Arg Ser Gly Asn Cys Val Pro  
                     35                    40                    45  
 5 Cys Asn Gln Cys Gly Pro Gly Met Glu Leu Ser Lys Glu Cys Gly Phe  
                     50                    55                    60  
 Gly Tyr Gly Glu Asp Ala Gln Cys Val Thr Cys Arg Leu His Arg Phe  
                     65                    70                    75                    80  
 Lys Glu Asp Trp Gly Phe Gln Lys Cys Lys Pro Cys Leu Asp Cys Ala  
 10                    85                    90                    95  
 Val Val Asn Arg Phe Gln Lys Ala Asn Cys Ser Ala Thr Ser Asp Ala  
                     100                    105                    110  
 Ile Cys Gly Asp Cys Leu Pro Gly Phe Tyr Arg Lys Thr Lys Leu Val  
                     115                    120                    125  
 15 Gly Phe Gln Asp Met Glu Cys Val Pro Cys Gly Asp Pro Pro Pro Pro  
                     130                    135                    140  
 Tyr Glu Pro His Cys Ala Ser Lys Val Asn Leu Val Lys Ile Ala Ser  
                     145                    150                    155                    160  
 Thr Ala Ser Ser Pro Arg Asp Thr Ala Leu Ala Ala Val Ile Cys Ser  
 20                    165                    170                    175  
 Ala Leu Ala Thr Val Leu Leu Ala Leu Leu Ile Leu Cys Val Ile Tyr  
                     180                    185                    190  
 Cys Lys Arg Gln Phe Met Glu Lys Lys Pro Ser Trp Ser Leu Arg Ser  
                     195                    200                    205  
 25 Gln Asp Ile Gln Tyr Asn Gly Ser Glu Leu Ser Cys Leu Asp Pro Arg

	210	215	220	
	Gln	Leu	His	Tyr
	Ala	His	Arg	Ala
	Cys	Cys	Gln	Cys
	Arg	Arg	Asp	
	225	230	235	240
	Ser	Val	Gln	Thr
	Cys	Gly	Pro	Val
	Arg	Leu	Leu	Pro
	Ser	Met	Cys	Cys
5	245	250	255	
	Glu	Glu	Ala	Cys
	Ser	Pro	Asn	Pro
	Ala	Thr	Leu	Gly
	Cys	Gly	Val	His
	260	265	270	
	Ser	Ala	Ala	Ser
	Leu	Gln	Ala	Arg
	Asn	Ala	Gly	Pro
	Ala	Gly	Glu	Met
	275	280	285	
10	Val	Pro	Thr	Phe
	Phe	Gly	Ser	Leu
	Thr	Gln	Ser	Ile
	Cys	Gly	Glu	Phe
	290	295	300	
	Ser	Asp	Ala	Trp
	Pro	Leu	Met	Gln
	Asn	Pro	Met	Gly
	Gly	Asp	Asn	Ile
	305	310	315	320
	Ser	Phe	Cys	Asp
	Ser	Tyr	Pro	Glu
	Leu	Thr	Gly	Glu
	Asp	Ile	His	Ser
15	325	330	335	
	Leu	Asn	Pro	Glu
	Leu	Glu	Ser	Ser
	Thr	Ser	Leu	Asp
	Ser	Asn	Ser	Ser
	340	345	350	
	Gln	Asp	Leu	Val
	Gly	Gly	Ala	Val
	Pro	Val	Gln	Ser
	His	Ser	Glu	Asn
	355	360	365	
20	Phe	Thr	Ala	Ala
	Thr	Asp	Leu	Ser
	Arg	Tyr	Asn	Asn
	Thr	Leu	Val	Glu
	370	375	380	
	Ser	Ala	Ser	Thr
	Gln	Asp	Ala	Leu
	Thr	Met	Arg	Ser
	Gln	Leu	Asp	Gln
	385	390	395	400
	Glu	Ser	Gly	Ala
	Ile	Ile	His	Pro
	Ala	Thr	Gln	Thr
	Ser	Leu	Gln	Val
25	405	410	415	

Arg Gln Arg Leu Gly Ser Leu

420

SEQ ID NO.: 6

5 Length: 1269 base pairs

Type: nucleic acid

Strandness: single

Topology: linear

Molecule type: cDNA to mRNA

10 Sequecne

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ATGGCTTTAA AAGTGCTACT AGAACAAGAG AAAACGTTTT TCACTCTTTT AGTATTACTA 60
GGCTATTTGT CATGTAAAGT GACTTGTGAA ACAGGAGACT GTAGACAGCA AGAATTCAGG 120
GATCGGTCTG GAAACTGTGT TCCCTGCAAC CAGTGTGGGC CAGGCATGGA GTTGTCTAAG 180
15 GAATGTGGCT TCGGCTATGG GGAGGATGCA CAGTGTGTGA CGTGCCGGCT GCACAGGTTT 240
AAGGAGGACT GGGGCTTCCA GAAATGCAAG CCCTGTCTGG ACTGCGCAGT GGTGAACCGC 300
TTTCAGAAGG CAAATTGTTC AGCCACCAGT GATGCCATCT GCGGGGACTG CTTGCCAGGA 360
TTTTATAGGA AGACGAAACT TGTCGGCTTT CAAGACATGG AGTGTGTGCC TTGTGGAGAC 420
CCTCCTCCTC CTTACGAACC GCACTGTGCC AGCAAGGTCA ACCTCGTGAA GATCGCGTCC 480
20 ACGGCCTCCA GCCCACGGGA CACGGCGCTG GCTGCCGTTA TCTGCAGCGC TCTGGCCACC 540
GTCCTGCTGG CCCTGCTCAT CCTCTGTGTC ATCTATTGTA AGAGACAGTT TATGGAGAAG 600
AAACCCAGCT GGTCTCTGCG GTCACAGGAC ATTCAGTACA ACGGCTCTGA GCTGTCGTGT 660
CTTGACAGAC CTCAGCTCCA CGAATATGCC CACAGAGCCT GCTGCCAGTG CCGCCGTGAC 720
TCAGTGCAGA CCTGCGGGCC GGTGCGCTTG CTCCCATCCA TGTGCTGTGA GGAGGCCTGC 780
25 AGCCCCAACC CGGCGACTCT TGGTTGTGGG GTGCATTCTG CAGCCAGTCT TCAGGCAAGA 840
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AACGCAGGCC CAGCCGGGGA GATGGTGCCG ACTTTCTTCG GATCCCTCAC GCAGTCCATC 900  
 TGTGGCGAGT TTTCAGATGC CTGGCCTCTG ATGCAGAATC CCATGGGTGG TGACAACATC 960  
 TCTTTTTGTG ACTCTTATCC TGAAGTCACT GGAGAAGACA TTCATTCTCT CAATCCAGAA 1020  
 CTTGAAAGCT CAACGTCTTT GGATTCAAAT AGCAGTCAAG ATTTGGTTGG TGGGGCTGTT 1080  
 5 CCAGTCCAGT CTCATTCTGA AAACCTTACA GCAGCTACTG ATTTATCTAG ATATAACAAC 1140  
 AACTGGTAG AATCAGCATC AACTCAGGAT GCACTAACTA TGAGAAGCCA GCTAGATCAG 1200  
 GAGAGTGGCG CTATCATCCA CCCAGCCACT CAGACGTCCC TCCAGGTAAG GCAGCGACTG 1260  
 GGTTCCTG 1269

10 SEQ ID NO.: 7

Length: 1496 base pairs

Type: nucleic acid

Strandness: single

Topology: linear

15 Molecule type: cDNA to mRNA

Sequence

GGGAACGTAG AACTCTCCAA CAATAAATAC ATTTGATAAG AAAGATGGCT TAAAAAGTGC 60  
 TACTAGAACA AGAGAAAACG TTTTCACTC TTTTAGTATT ACTAGGCTAT TTGTCATGTA 120  
 20 AAGTGACTTG TGAAACAGGA GACTGTAGAC AGCAAGAATT CAGGGATCGG TCTGGAAACT 180  
 GTGTTCCCTG CAACCAAGTGT GGGCCAGGCA TGGAGTTGTC TAAGGAATGT GGCTTCGGCT 240  
 ATGGGGAGGA TGCACAGTGT GTGACGTGCC GGCTGCACAG GTTCAAGGAG GACTGGGGCT 300  
 TCCAGAAATG CAAGCCCTGT CTGGACTGCG CAGTGGTGAA CCGCTTTCAG AAGGCAAATT 360  
 GTTCAGCCAC CAGTGATGCC ATCTGCGGGG ACTGCTTGCC AGGATTTTAT AGGAAGACGA 420  
 25 AACTTGTCGG CTTTCAAGAC ATGGAGTGTG TGCCTTGTGG AGACCCTCCT CCTCCTTACG 480

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AACCGCACTG TGCCAGCAAG GTCAACCTCG TGAAGATCGC GTCCACGGCC TCCAGCCCAC 540
GGGACACGGC GCTGGCTGCC GTTATCTGCA GCGCTCTGGC CACCGTCCTG CTGGCCCTGC 600
TCATCCTCTG TGTATCTAT TGTAAGAGAC AGTTTATGGA GAAGAAACCC AGCTGGTCTC 660
TGCGGTCACA GGACATTCAG TACAACGGCT CTGAGCTGTC GTGTCTTGAC AGACCTCAGC 720
5 TCCACGAATA TGCCACAGA GCCTGCTGCC AGTGCCGCCG TGAATCAGTG CAGACCTGCG 780
GGCCGGTGCG CTTGCTCCCA TCCATGTGCT GTGAGGAGGC CTGCAGCCCC AACCCGGCGA 840
CTCTTGGTTG TGGGGTGCAT TCTGCAGCCA GTCTTCAGGC AAGAAACGCA GGCCCAGCCG 900
GGGAGATGGT GCCGACTTTC TTCGGATCCC TCACGCAGTC CATCTGTGGC GAGTTTTTCAG 960
ATGCCTGGCC TCTGATGCAG AATCCCATGG GTGGTGACAA CATCTCTTTT TGTGACTCTT 1020
10 ATCCTGAACT CACTGGAGAA GACATTCATT CTCTCAATCC AGAACTTGAA AGCTCAACGT 1080
CTTTGGATTC AAATAGCAGT CAAGATTTGG TTGGTGGGGC TGTTCAGTC CAGTCTCATT 1140
CTGAAAACCTT TACAGCAGCT ACTGATTTAT CTAGATATAA CAACACACTG GTAGAATCAG 1200
CATCAACTCA GGATGCACTA ACTATGAGAA GCCAGCTAGA TCAGGAGAGT GGCCTATCA 1260
TCCACCCAGC CACTCAGACG TCCCTCCAGG TAAGGCAGCG ACTGGGTTCC CTGTGAACAC 1320
15 AGCACTGACT TACAGTAGAT CAGAACTCTG TTCCAGCAT AAGATTTGGG GGAACCTGAT 1380
GAGTTTTTTT TTTGCATCTT TAATAATTTT TTGTATGTTG TAGAGTATGT TTTAAATAA 1440
ATTTCAAGTA TTTTTTTTAA AACTAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAA 1496

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SEQ ID NO.: 8

20 Length: 1496 base pairs

Type: nucleic acid

Strandness: single

Topology: linear

Molecule type: cDNA to mRNA

25

Original source

Organism: Homo Sapiens

Cell line: HAS303

Feature

5 Name/Key: CDS

Location: 45..1313

Identification method: P

Name/Key: sig peptide

10 Location: 45..119

Identification method: S

Name/Key: mat peptide

Location: 120..1313

Identification method: S

15 Sequence

GGGAACGTAG AACTCTCCAA CAATAAATAC ATTTGATAAG AAAG ATG GCT TTA AAA 56

Met Ala Leu Lys

-25

20 GTG CTA CTA GAA CAA GAG AAA ACG TTT TTC ACT CTT TTA GTA TTA CTA 104

Val Leu Leu Glu Gln Glu Lys Thr Phe Phe Thr Leu Leu Val Leu Leu

-20

-15

-10

GGC TAT TTG TCA TGT AAA GTG ACT TGT GAA ACA GGA GAC TGT AGA CAG 152

Gly Tyr Leu Ser Cys Lys Val Thr Cys Glu Thr Gly Asp Cys Arg Gln

25 -5

1

5

10

	CAA GAA TTC AGG GAT CGG TCT GGA AAC TGT GTT CCC TGC AAC CAG TGT	200
	Gln Glu Phe Arg Asp Arg Ser Gly Asn Cys Val Pro Cys Asn Gln Cys	
	15 20 25	
	GGG CCA GGC ATG GAG TTG TCT AAG GAA TGT GGC TTC GGC TAT GGG GAG	248
5	Gly Pro Gly Met Glu Leu Ser Lys Glu Cys Gly Phe Gly Tyr Gly Glu	
	30 35 40	
	GAT GCA CAG TGT GTG ACG TGC CGG CTG CAC AGG TTC AAG GAG GAC TGG	296
	Asp Ala Gln Cys Val Thr Cys Arg Leu His Arg Phe Lys Glu Asp Trp	
	45 50 55	
10	GGC TTC CAG AAA TGC AAG CCC TGT CTG GAC TGC GCA GTG GTG AAC CGC	344
	Gly Phe Gln Lys Cys Lys Pro Cys Leu Asp Cys Ala Val Val Asn Arg	
	60 65 70 75	
	TTT CAG AAG GCA AAT TGT TCA GCC ACC AGT GAT GCC ATC TGC GGG GAC	392
	Phe Gln Lys Ala Asn Cys Ser Ala Thr Ser Asp Ala Ile Cys Gly Asp	
15	80 85 90	
	TGC TTG CCA GGA TTT TAT AGG AAG ACG AAA CTT GTC GGC TTT CAA GAC	440
	Cys Leu Pro Gly Phe Tyr Arg Lys Thr Lys Leu Val Gly Phe Gln Asp	
	95 100 105	
	ATG GAG TGT GTG CCT TGT GGA GAC CCT CCT CCT CCT TAC GAA CCG CAC	488
20	Met Glu Cys Val Pro Cys Gly Asp Pro Pro Pro Pro Tyr Glu Pro His	
	110 115 120	
	TGT GCC AGC AAG GTC AAC CTC GTG AAG ATC GCG TCC ACG GCC TCC AGC	536
	Cys Ala Ser Lys Val Asn Leu Val Lys Ile Ala Ser Thr Ala Ser Ser	
	125 130 135	
25	CCA CGG GAC ACG GCG CTG GCT GCC GTT ATC TGC AGC GCT CTG GCC ACC	584



	Pro Arg Asp Thr Ala Leu Ala Ala Val Ile Cys Ser Ala Leu Ala Thr	
	140                      145                      150                      155	
	GTC CTG CTG GCC CTG CTC ATC CTC TGT GTC ATC TAT TGT AAG AGA CAG	632
	Val Leu Leu Ala Leu Leu Ile Leu Cys Val Ile Tyr Cys Lys Arg Gln	
5	160                      165                      170	
	TTT ATG GAG AAG AAA CCC AGC TGG TCT CTG CGG TCA CAG GAC ATT CAG	680
	Phe Met Glu Lys Lys Pro Ser Trp Ser Leu Arg Ser Gln Asp Ile Gln	
	175                      180                      185	
	TAC AAC GGC TCT GAG CTG TCG TGT CTT GAC AGA CCT CAG CTC CAC GAA	728
10	Tyr Asn Gly Ser Glu Leu Ser Cys Leu Asp Rro Arg Gln Leu His Glu	
	190                      195                      200	
	TAT GCC CAC AGA GCC TGC TGC CAG TGC CGC CGT GAC TCA GTG CAG ACC	776
	Tyr Ala His Arg Ala Cys Cys Gln Cys Arg Arg Asp Ser Val Gln Thr	
	205                      210                      215	
15	TGC GGG CCG GTG CGC TTG CTC CCA TCC ATG TGC TGT GAG GAG GCC TGC	824
	Cys Gly Pro Val Arg Leu Leu Pro Ser Met Cys Cys Glu Glu Ala Cys	
	220                      225                      230                      235	
	AGC CCC AAC CCG GCG ACT CTT GGT TGT GGG GTG CAT TCT GCA GCC AGT	872
	Ser Pro Asn Pro Ala Thr Leu Gly Cys Gly Val His Ser Ala Ala Ser	
20	240                      245                      250	
	CTT CAG GCA AGA AAC GCA GGC CCA GCC GGG GAG ATG GTG CCG ACT TTC	920
	Leu Gln Ala Arg Asn Ala Gly Pro Ala Gly Glu Met Val Pro Thr Phe	
	255                      260                      265	
	TTC GGA TCC CTC ACG CAG TCC ATC TGT GGC GAG TTT TCA GAT GCC TGG	968
25	Phe Gly Ser Leu Thr Gln Ser Ile Cys Gly Glu Phe Ser Asp Ala Trp	

	270	275	280	
	CCT CTG ATG CAG AAT CCC ATG GGT GGT GAC AAC ATC TCT TTT TGT GAC	1016		
	Pro Leu Met Gln Asn Pro Met Gly Gly Asp Asn Ile Ser Phe Cys Asp			
	285	290	295	
5	TCT TAT CCT GAA CTC ACT GGA GAA GAC ATT CAT TCT CTC AAT CCA GAA	1064		
	Ser Tyr Pro Glu Leu Thr Gly Glu Asp Ile His Ser Leu Asn Pro Glu			
	300	305	310	315
	CTT GAA AGC TCA ACG TCT TTG GAT TCA AAT AGC AGT CAA GAT TTG GTT	1112		
	Leu Glu Ser Ser Thr Ser Leu Asp Ser Asn Ser Ser Gln Asp Leu Val			
10	320	325	330	
	GGT GGG GCT GTT CCA GTC CAG TCT CAT TCT GAA AAC TTT ACA GCA GCT	1160		
	Gly Gly Ala Val Pro Val Gln Ser His Ser Glu Asn Phe Thr Ala Ala			
	335	340	345	
	ACT GAT TTA TCT AGA TAT AAC AAC ACA CTG GTA GAA TCA GCA TCA ACT	1208		
15	Thr Asp Leu Ser Arg Tyr Asn Asn Thr Leu Val Glu Ser Ala Ser Thr			
	350	355	360	
	CAG GAT GCA CTA ACT ATG AGA AGC CAG CTA GAT CAG GAG AGT GGC GCT	1256		
	Gln Asp Ala Leu Thr Met Arg Ser Gln Leu Asp Gln Glu Ser Gly Ala			
	365	370	375	
20	ATC ATC CAC CCA GCC ACT CAG ACG TCC CTC CAG GTA AGG CAG CGA CTG	1304		
	Ile Ile His Pro Ala Thr Gln Thr Ser Leu Gln Val Arg Gln Arg Leu			
	380	385	390	395
	GGT TCC CTG TGAACACAG CACTGACTTA CAGTAGATCA GAACTCTGTT CCCAGCATAA	1362		
	Gly Ser Leu			
25	GATTTGGGGG AACCTGATGA GTTTTTTTTT TGCATCTTTA ATAATTTCTT GTATGTTGTA	1422		

GAGTATGTTT TAAAATAAAT TTCAAGTATT TTTTAAAA ACTAAAAAA AAAAAAAAA 1482

AAAAAAAAA AAAA

1496

SEQ ID NO.: 9

5 Length: 35 base pairs

Type: nucleic acid

Strandness: single

Topology: linear

Sequence

10

CGATTGAATT CTAGACCTGC CTCGAGNNNN NNNNN

SEQ ID NO.: 10

Length: 28 base pairs

15 Type: nucleic acid

Strandness: single

Topology: linear

Sequence

AGAAAGATGG CTTTAAAAGT GCTACTAG